



Published in final edited form as:

*J Anal Toxicol.* 2015 June ; 39(5): 347–352. doi:10.1093/jat/bkv016.

## Determination of Toxic Metals in Little Cigar Tobacco with “Triple Quad” ICP-MS

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### Abstract

Smoking remains the leading cause of preventable death in the United States. Much of the focus on harmful constituents (HPHCs) in tobacco products has been on cigarettes. Little cigars have gained popularity over the last decade as tobacco taxes made cigarettes more expensive in the U.S. Many little cigar brands are similar in size with cigarettes and may be smoked in a similar manner.

Scant data are available on HPHC levels in little cigars, therefore we developed and applied a new analytical method to determine concentrations of ten toxic metals in little cigar tobacco. The method utilizes “triple quadrupole” ICP-MS. By optimizing octapole bias, energy discrimination, and cell gas flow settings, we were able to accurately quantify a range of elements including those for which the cell gas reactions were endothermic. All standard modes (Single Quad No Gas, MS/MS NH<sub>3</sub>/He, and MS/MS O<sub>2</sub>) were utilized for the quantitation of ten toxic metals in little cigar tobacco, including uranium, which was added as an analyte in the new method.

Because of the elimination of interfering ions at “shifted analyte masses,” detection limits were lower compared to a previous method. Tobacco selenium concentrations were below the limit of detection in the previous method, but the new technology made it possible to report all selenium concentrations.

### Introduction

Cigarette smoking is estimated to cause more than 480,000 deaths annually, including deaths from secondhand smoke (1). Smoking prevalence has fallen in the U.S. since its peak in 1963, but has remained at almost 20% of the U.S. population between 2004 and 2010 (2). In 2012, adult smoking remained highest among those had completed high school education or less or who were below poverty level income (3).

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#### Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

A recent follow up on the Virginia Youth Tobacco Survey found that approximately the same number of adolescents (9.2%) reported smoking Black and Mild® little cigars (which are typically smoked as cigarettes) as the number that reported smoking cigarettes (9.2%), though only 6.1% reported using cigars as a category of tobacco product (4). This data indicated that many of the youth surveyed understood smoking little cigars as equivalent to smoking cigarettes, a distinction based on tobacco wrapping for little cigars versus wood pulp paper wrapping for cigarettes. This minor difference that determines the product category is apparently not a distinguishing factor to many little cigar smokers. The authors of the manuscript concluded that little cigar smoking among adolescents had been underreported, because they perceived smoking little cigars as equivalent to smoking cigarettes (4). The strong similarity between little cigars and cigarettes is consistent with the marketed design of little cigars (5). Indeed, 42.4% of smokers surveyed in the 2011 National Youth Tobacco Survey responded that they used flavored cigars or flavored cigarettes (6).

An analysis of data from the 2012–2013 National Adult Tobacco Survey found that approximately one in 20 U.S. adults smoke cigars. Among adults who smoke cigars, 18.4% smoke little filtered cigars. The groups that more often chose to smoke little cigars were age 50 to 64, women, white non-hispanic, with high school education level or lower, and with income in the lowest bracket (7).

The established health risks from tobacco products, the current, new regulatory environment, and the wide diversity of commercial tobacco products available in the U.S. necessitate the development and continued improvement of robust, high throughput methods for tobacco analysis in order to quantitatively determine concentrations of toxic substances, particularly metals, found in tobacco products.

A risk assessment approach that identified 5 major classes of toxic constituents of tobacco smoke found that toxic metals impart carcinogenic health risk second only to the group of small molecules and aldehydes (8). Metals in tobacco smoke are likely candidate agents for increased risk of cancer and diseases that are consequences of chronic inflammation and sensitization such as COPD and asthma (8,9). Thus, characterization of metals in tobacco filler is necessary to fill an outstanding research gap and provide data to help assess potential health impact from little cigars.

We have previously reported concentrations of ten toxic and carcinogenic metals in tobacco filler from 50 domestic cigarette varieties (10). Mainstream smoke toxic metal deliveries in a subset of domestic cigarette brands (11,12) and from counterfeit cigarettes seized in the U.S. have been previously reported by our laboratory (13). Multivariate statistical analysis of the tobacco and smoke data correlated cigarette design and physical parameters with delivery of arsenic, cadmium, and lead from the filler tobacco into mainstream smoke (12). Our multivariate statistical analyses showed that the transfer of arsenic ( $p < 0.0001$ ), cadmium ( $p < 0.0001$ ), and lead ( $p = 0.0011$  [ISO regimen],  $p = 0.0002$  Intense regimen) into mainstream cigarette smoke was strongly positively correlated with tobacco mass (12) in both ISO (14) and Health Canada Intense (15) smoking regimens. Though measurements of toxic metal concentrations in tobacco smoke are a major goal, data suggest smoke concentrations are highly dependent on the tobacco lamina metal concentrations and the

total tobacco mass in the cigarette (12,16). Therefore determination of the unsmoked tobacco metals content provides important information to be used in conjunction with metal levels in smoke. The harmful and potentially harmful constituents (HPHCs) of little cigar products have been understudied, therefore we focused on inorganic HPHCs in the tobacco of select brands of little cigars.

We report using a new approach in “triple quad” ICP-MS technology for quantitative determination of toxic metals in little cigar tobacco. When used in MS/MS or “Triple Quad” mode, the instrument employs mass exclusion with single mass unit bandpass resolution in the first quadrupole. The exclusion of all masses except the mass of the analyte isotope is followed by reaction of the analyte with a gas in the octapole cell. When oxygen is used as the reactive gas, a 16 mass unit shift of the analyte mass results from formation of the ion oxide. MS/MS mode may also be used with ammonia in the octapole cell (10% ammonia, 90% helium), which results in mass unit shifts of multiples of 17 to form oligoammino cluster ion complexes. The mass shifts result in a more optimal quantitation of the analyte at masses where discrimination against interfering ions in the first quadrupole has occurred. Utilization of this analyte “mass shift” uniquely eliminates potential interfering ions at the new mass, allowing improved analyte quantification. Background at the analyzed mass as a consequence is very low compared to analysis with a single quadrupole instrument. We are not aware of any previous applications in which the “Triple Quad” technology in two distinct gas modes has been employed along with the single quad mode for the analysis of ten toxic metal analytes in tobacco.

## Experimental

### Little cigar samples

Little cigar samples were purchased domestically in 2014. The samples were selected according to a convenience sampling plan based on brand availability, assigned unique identification numbers, and logged into a database. Samples were stored in their original packaging until needed. Only authorized personnel had access to the samples.

### Little cigar tobacco sample and blank preparation for analysis

Tobacco filler samples from little cigars were dried in perfluoroalkoxy (PFA) vials for a minimum of 1 hour at 90°C. Dried tobacco was rendered more homogeneous by grinding for 20 seconds in a Smart Grind coffee grinder (Black and Decker, Middleton, WI, USA). (ThermoFisher, Pittsburgh, PA, USA). Samples were tightly sealed until weighed for analysis.

Weighed sample portions (0.100 to 0.150 g dried tobacco) were prepared for analysis by microwave digestion in Tracecleaned 75 mL TFM vessels with a Milestone Ethos microwave system with Pro-24 rotor (Milestone, Shelton, CT, USA). Digestion was accomplished with double distilled nitric and hydrofluoric acids (9.0 mL and 0.5 mL, respectively, GFS, Powell, OH, USA), and 0.5 mL Tamapure AA-10 35% hydrogen peroxide (Moses Lake Industries, Moses Lake, WA, USA) in each vessel. Procedural blanks were prepared by addition of all reagents to a vessel without tobacco before heating in the microwave system. The microwave heating profile was programmed to start at ambient

temperature and increase to 190°C in 10 minutes, followed by a 15 minute digestion at 190°C. Digested samples were diluted with approximately 40 mL ultrapure water and transferred into acid-cleaned 100.0 mL class A polymethylpentene volumetric flasks. Additional ultrapure water rinses of the digestion vessel were used to quantitatively transfer the digested material and bring the volume to the 100.0 mL mark in the volumetric flask. Procedural blanks were transferred to volumetric flasks and diluted in the same manner as tobacco samples. The microwave system and the digestion method represent modifications to our previously published analytical methods (10,17).

### ICP-MS Quantification

Five calibration standard solutions were prepared by dilution of High Purity Standards (HPS, Charleston, SC, USA) and National Institute for Standards and Technology lead standard SRM 981 (NIST, Gaithersburg, MD, USA). The calibration standard solutions were prepared in 9% v/v nitric acid and 0.5% hydrofluoric acid to match the diluted acid digest concentrations. The concentration ranges for the respective standards were 0.005 to 0.250 µg/L (beryllium), 0.0100 to 0.500 µg/L (uranium), 0.0500 to 2.50 µg/L (arsenic and selenium), 0.100 to 5.00 µg/L (cobalt), 0.200 to 10.00 µg/L (chromium and lead), 0.500 to 25.00 µg/L nickel and cadmium), and 20.0 to 1000.0 µg/L (manganese). Calibration ranges for all metals spanned the observed levels in domestic cigarette filler tobacco (10). Internal standard solutions were prepared by dilution of HPS rhodium, tellurium, iridium, and thulium standards. The internal standards (20.0 µg/L Rh and Te, 2.0 µg/L Tm and Ir) were prepared in 1% v/v nitric acid and 2% v/v 2-propanol (semiconductor grade, Sigma-Aldrich, St. Louis, MO, USA).

Instrumentation included an SC-DX FAST autosampler (Elemental Scientific, Omaha, NE, USA) for high sample throughput and elimination of carryover, and an Agilent 8800 “Triple Quad” ICP-MS (Tokyo, Japan). The introduction system was the standard Agilent Peltier-cooled PFA spray chamber and nebulizer, sapphire injector, used with Pt-tipped sampler and Pt-tipped nickel skimmer cone (Spectron, Ventura, CA, USA). The internal standard and the sample solutions from the FAST loop were introduced simultaneously via the peristaltic pump at 0.45 rps with 0.44 mm i.d. PVC pump tubing from each channel teed together for a further 1+1 dilution of samples prior to introduction. Analyses were optimized at 1550 W forward power and 0.90 L/min carrier gas flow with no dilution or makeup gas. Signal was maximized while maintaining 0.9 to 1.1% CeO/Ce during tuning. Octapole bias was optimized at -7.0 V with 6.0 V Energy Discrimination in “Single Quad” (no cell gas) mode. Octapole bias was optimized at -18.0 V for optimum cell gas reactions and ion transmission with -8.0 V Energy Discrimination in MS/MS mode. Sampling depth and lens parameters were optimized for highest signal and optimum peak shape while maintaining low oxides. Details regarding instrument mode, cell gas, and assignment of internal standards are included in Table 1.

In MS/MS Ammonia mode, 10% NH<sub>3</sub> / 90% He was introduced into the octapole cell at 6 mL/min. In MS/MS Oxygen mode, O<sub>2</sub> was introduced into the octapole cell at 0.6 mL/min.

### Quality control and validation procedures

Reference tobacco 1S3 (North Carolina State University, Raleigh, NC, USA) and Certified Reference Material (CRM) CTA-OTL-1 (Instytut Chemii i Techniki J drowej, Warszawa, Poland) were used as quality control materials. Quality control (QC) samples were prepared for the beginning (post-calibration) and end of each analytical run. The analytical QC samples were evaluated using a modified Westgard evaluation approach (18) with SAS software (Cary, NC, USA). When a QC analyte was determined to be out of control according to the modified Westgard criteria, results for the failed analyte in the respective batch were not used and analyses were repeated. Additional CRMs, INCT-OBTL-5, INCT-PVTL-6 (Instytut Chemii i Techniki J drowej, Warszawa, Poland), and SRMs 1570a (Spinach Leaves), and 1573a (Tomato Leaves) (NIST, Gaithersburg, MD, USA) were analyzed to establish validation of accuracy.

### Lowest reportable levels

The Procedural Detection Limits (LODs) were statistically adjusted for potential false positive and false negative overlap and determined using the following equation (19).

$$\text{LOD} = [\text{Mean}_{\text{procedural blank}} + 1.645 * (\text{S}_{\text{procedural blank}} + B)] / (1 - 1.645 * A)$$

$\text{Mean}_{\text{procedural blank}}$  and  $\text{S}_{\text{procedural blank}}$  were determined as the mean and standard deviation from analyses of 20 procedural digest blanks. Factors A (slope) and B (intercept) were determined according to Taylor (20), by plotting standard deviations for the procedural blank, and digests of 1S3, CTA-OTL-1, INCT-PVTL-6, NIST SRM 1570a (Spinach Leaves), and NIST SRM 1573a (Tomato Leaves) versus their mean concentrations over 20 analytical runs.

The Lowest Reportable Concentration Level (LRL) was chosen from the higher of the adjusted LOD, or the concentration of the lowest calibration standard expressed in terms of  $\mu\text{g/g}$ , whichever was higher.

### Data comparisons with cigarette tobacco

Statistical comparisons of toxic metal concentrations between cigarette and little cigar tobacco were performed using t test (Excel®, Microsoft, Redmond, WA, USA).

## Results and discussion

### High throughput sample preparations

The Milestone digestion system is not sequential as was the system that we have previously used (10). This system was chosen because it has a 24 position rotor for TFM vessels, enabling digestion of 24 samples per digest rather than 11 samples with a previous system (17); and because this system is more compatible with hydrofluoric acid. We were concerned about pouring sample digests containing HF over the quartz lip of the digestion vessel used in the sequential system during transfers to the volumetric flasks. We had experimented with adding HF post-digest with good results for most analytes; and the addition of HF either pre-digest or post-digest was effective for dissolution of silicates. However, when yields were verified by isotope dilution, we noticed low reference material

yields particularly for uranium and chromium unless HF was added pre-digest rather than post-digest. When we performed digestions that included HF, no silicate precipitates were observed in diluted samples; the samples were colorless, indicating complete digestion; and chromium and uranium yields were quantitative. The SC-DX FAST rinse-out autosampler only required 30 seconds processing time between samples as compared with approximately 90 seconds for the Agilent ISIS system (12). The use of a larger capacity microwave rotor and replacement of the autosampler system improved our throughput compared to previously published methods (10,17).

### Cell gas optimizations

Since there were negligible interferences for  $^9\text{Be}$ , Pb isotopes, and  $^{238}\text{U}$ , these were analyzed in “Single Quad” (no cell gas) mode, in which quad 1 is used only as an ion focusing guide. This mode may impart greater sensitivity for isotopes that do not have significant interferences; and it is essential for  $^9\text{Be}^+$  because of the significant loss of kinetic energy and increased scattering losses by low mass ions via collisions with added gases. Our initial investigations into whether to use oxygen for the cell gas for other analytes were in line with gas phase reaction enthalpies for the respective analytes (21).  $^{111}\text{Cd}$  was analyzed in oxygen mode without mass shift. Reaction of  $^{111}\text{Cd}^+$  to form  $^{111}\text{CdO}^+$  was negligible, while the native  $^{95}\text{MoO}^+$  interference quantitatively reacted with  $\text{O}_2$  to form  $^{95}\text{MoO}_2^+$  at oxygen flow rates 0.5 mL/min.  $^{75}\text{As}^+$  also reacted quantitatively with  $\text{O}_2$  at flow rates 0.5 mL/min. Though the reactions to form  $^{55}\text{MnO}^+$ ,  $^{60}\text{NiO}^+$ , and  $^{80}\text{SeO}^+$  were endothermic and not quantitative, these elements reacted sufficiently for optimum analysis in oxygen mode. Reactions went further to completion at higher  $\text{O}_2$  flow rates, though total metal oxide ion transmission declined, possibly as a consequence of loss kinetic energy or ion scattering due to increased the numbers of collisions with the cell gas at higher flow rates. The optimum 0.6 mL/min  $\text{O}_2$  flow rate was derived as a compromise between higher oxide conversions and suppressed ion counts at higher flow rates.

At 90% He/10%  $\text{NH}_3$  flow rates of 5 to 7 mL/min,  $^{52}\text{Cr}^+$  and  $^{59}\text{Co}^+$  reacted with  $\text{NH}_3$  to form predominantly the diammine cluster ion complexes. The  $^{103}\text{Rh}$  internal standard formed a mix of polyammine cluster ions dependent on the He/ $\text{NH}_3$  flow rates. At the 0.6 mL/min flow rate, the tetrammine complex was predominant.  $\text{Cr}^+$  formed approximately 37% diammine complex at 6 mL/min He/ $\text{NH}_3$  versus 0.8% obtained with lower gas flow and less negative octapole bias settings.<sup>21</sup>  $\text{Co}^+$  formed approximately 51% diammine complex at 6 mL/min He/ $\text{NH}_3$  versus 0.8% obtained under less optimum conditions.<sup>22</sup> Much of the remaining  $\text{Co}^+$  formed the triammine complex.  $\text{Ni}^+$  formed predominantly the triammine complex under these conditions, but better sensitivity was obtained when it was mass shifted in oxygen mode.  $\text{Rh}^+$  formed predominantly the tetrammine complex at 6 mL/min He/ $\text{NH}_3$ . A flow rate of 6 mL/min He/ $\text{NH}_3$  resulted as a compromise between higher cluster ion complex formation and suppressed ion counts at higher flow rates. Formation of mono-, and tetraammine complex ions was less than 4% for  $\text{Cr}^+$  and  $\text{Co}^+$  utilizing the gas flow rates, octapole bias, and KED voltage settings described above.

Comparison of LODs utilizing the MS/MS mode technology with LODs for analytes common to former methods (10,17, Table 2) shows that five of six LODs, though more



rigorously calculated in the present work, were lower for analytes for which the mass analyzed had been shifted from the analyte isotopic mass 16 units by addition of O from O<sub>2</sub> (As, Mn, Se; with Ni being the only exception) or 34 units by addition of 2NH<sub>3</sub> (Co, Cr). This results in part from elimination of interferences in the first quadrupole at the cluster ion mass. The LOD was also lower for <sup>111</sup>Cd, for which the minor interference from <sup>95</sup>Mo<sup>16</sup>O<sup>+</sup> was shifted to mass 127 (<sup>95</sup>Mo<sup>16</sup>O<sub>2</sub><sup>+</sup>). A striking benefit of this technology is that all tobacco selenium concentrations were above the LRL in this report, whereas all were <LOD previously (10).

### Accuracy

The method accuracy was assessed by comparison of our new method's results with SRM and characterized CRM results (Table 3). Comparison of our results with certified, reference, and information values for NIST SRMs 1570a and 1573a (Spinach and Tomato Leaves, respectively) demonstrated good accuracy even when the concentration target ranges were close to or below the method LRLs, such as was the case for As and Pb (1570a), and Se (1573a). The only exception was a selenium result that was significantly below one (CTA-OTL1) of the five certified materials (good agreement for SE was observed for the other 4 reference materials; the reason for the discrepancy with Se for CTA-OTL1 is unknown). Comparison of our results with other SRM results, CRM results, and with our own previously published results (10) obtained with different instrumentation were also very favorable (Table 3).

### Analysis of results

The primary goals of this work were to validate a new multielement method for analysis of toxic metals in tobacco based on "triple quadrupole" ICP-MS technology, and to apply the method to analyses for an overview of the concentration levels for select toxic and carcinogenic metals in tobacco from little cigars. Because cigar tobacco is an agriculturally based product, and growing locations and conditions can influence metals uptake (23), the levels reported here relate to the little cigars analyzed in this study (Table 4).

The importance of monitoring the concentrations of various toxicants present in tobacco becomes increasingly important for multiple applications: counterfeit detection, assessment of health risks, or for regulatory efforts related to tobacco products. Because information is lacking on metals in little cigar products, accurate data is critical for educating the public on the hazards of tobacco use and for informing potential public health or regulatory decisions related to reducing or eliminating exposure risks to toxicants. For this study, we quantitatively analyzed 17 different little cigar brands purchased in 2014, using a novel "triple quadrupole" ICP-MS technology. All measurements were made in a strict QA/QC approach and the reported levels are an average obtained from pentuplicate (n=5) individual measurements of select metals (Table 4).

The mean concentration ranges for beryllium, chromium, manganese, nickel, arsenic, cadmium, and lead in little cigar tobacco were significantly wider than the ranges reported previously in cigarette tobacco. Uranium concentrations in tobacco were not previously reported. Cobalt was the only analyte with the mean concentration range in little cigar

tobacco slightly narrower than in cigarette tobacco (10). Despite wider concentration ranges, beryllium, chromium, manganese, cobalt, arsenic, selenium, cadmium, and lead concentrations in tobacco from little cigars were not significantly different from concentrations previously determined in cigarette tobacco (10) ( $p>0.05$ ). Nickel concentrations were significantly different from nickel concentrations in cigarette tobacco ( $p<0.01$ ). The mean nickel concentration in little cigar tobacco ( $2.56\text{ }\mu\text{g/g}$ ) was lower than the mean nickel concentration in cigarette tobacco ( $3.23\text{ }\mu\text{g/g}$ ) on a per gram basis.

Though the differences between overall mean toxicant concentrations in little cigar and cigarette filler tobacco, with the exception of nickel, were not significantly different, mean values for some brands were outside the corresponding ranges reported for cigarette filler tobacco (10). Mean nickel concentrations in tobacco from Cheyenne, and mean chromium, manganese, and nickel concentrations in tobacco from Hav-A-Tampa little cigars, for example, were above the respective ranges previously reported in cigarette filler tobacco.<sup>10</sup> Tobacco from several little cigars were lower in mean arsenic concentrations than reported in cigarette filler, whereas tobacco from Cheyenne little cigars had higher mean arsenic concentration than reported in cigarette filler tobacco (10). The Smokers Best and Santa Fe little cigar tobacco cadmium concentrations were lower than reported in cigarette tobacco filler, whereas the cadmium concentrations in tobacco from Al Capone, Captain Black, and Winchester little cigars were at or near the highest mean cadmium concentrations reported in cigarette filler tobacco (10). Tobacco from Remington and Winchester little cigars had lower mean lead concentrations than the range of mean concentrations reported for cigarette tobacco filler, whereas Swisher Sweets had the highest mean concentrations of lead and beryllium than in tobacco from other little cigars reported here, as well as from tobacco from 50 cigarettes previously reported (10). Thus, in general, the levels of toxic metals in little cigar tobacco span the ranges of mean concentrations previously determined in cigarette filler tobacco. Uranium concentrations in tobacco were not previously reported; and selenium was below previously reported limits of detection. Though selenium is a respiratory irritant, it is not known whether it contributes to tobacco product toxicity at the concentrations reported here. However both metals merit monitoring as selenium concentrations are required to be reported in tobacco products by some jurisdictions including the Canada Department of Justice Tobacco Reporting Regulations (24); and both uranium and selenium are in the U.S. Food and Drug Administration list of Harmful and Potentially Harmful Constituents (25).

## Conclusions

The method for analysis of toxic metals in tobacco reported here was developed utilizing new “triple quadrupole” ICP-MS technology. This is the first application of this technology to the analysis of 10 toxic metals in tobacco or similar matrices in three instrument modes, two of which utilize the instrument’s mass shift capabilities to avoid mass isobaric interferences. The method has been operated within an established QA/QC environment; and has been validated using tobacco reference materials and standard reference materials in related matrices.



The method was applied to the analysis of ten toxic metals in the tobacco filler of little cigars. Though the number of samples was limited, there were no significant differences in concentrations of toxic metals in tobacco from little cigars compared with concentrations in tobacco from cigarettes with the exception of nickel. We conclude that the mass shift approach offered by “triple quad” ICP-MS is highly effective at improving routine quantification of metals in tobacco by eliminating common interferences for the selected analytes.

## Acknowledgments

This study was funded by an interagency agreement by the U.S. Food and Drug Administration Center for Tobacco Products.

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**Table 1**

Instrument modes and internal standard assignments

Element, Isotope	Instrument Mode	Cell Gas	Quantitated Ion	Quantitated Mass	Internal Standard
<sup>9</sup> Be	Single Quad	None	<sup>9</sup> Be <sup>+</sup>	9	<sup>193</sup> Ir <sup>+</sup>
<sup>204+206+207+208</sup> Pb	Single Quad	None	<sup>204, 206, 207, 208</sup> Pb <sup>+</sup>	204 + 206 + 207 + 208	<sup>193</sup> Ir <sup>+</sup>
<sup>238</sup> U	Single Quad	None	<sup>238</sup> U <sup>+</sup>	238	<sup>169</sup> Tm <sup>+</sup>
<sup>52</sup> Cr	MS/MS	NH <sub>3</sub>	<sup>52</sup> Cr(NH <sub>3</sub> ) <sub>2</sub> <sup>+</sup>	86	<sup>103</sup> Rh(NH <sub>3</sub> ) <sub>4</sub> <sup>+</sup>
<sup>59</sup> Co	MS/MS	NH <sub>3</sub>	<sup>59</sup> Co(NH <sub>3</sub> ) <sub>2</sub> <sup>+</sup>	93	<sup>103</sup> Rh(NH <sub>3</sub> ) <sub>4</sub> <sup>+</sup>
<sup>55</sup> Mn	MS/MS	O <sub>2</sub>	<sup>55</sup> MnO <sup>+</sup>	71	<sup>103</sup> RhO <sup>+</sup>
<sup>60</sup> Ni	MS/MS	O <sub>2</sub>	<sup>60</sup> NiO <sup>+</sup>	76	<sup>103</sup> RhO <sup>+</sup>
<sup>75</sup> As	MS/MS	O <sub>2</sub>	<sup>75</sup> AsO <sup>+</sup>	91	<sup>125</sup> TeO <sup>+</sup>
<sup>80</sup> Se	MS/MS	O <sub>2</sub>	<sup>80</sup> SeO <sup>+</sup>	96	<sup>125</sup> TeO <sup>+</sup>
<sup>111</sup> Cd	MS/MS	O <sub>2</sub>	<sup>111</sup> Cd <sup>+</sup>	111	<sup>193</sup> Ir <sup>+</sup>

**Table 2**

Method LODs and LRLs

Analyte	Previously Reported LOD <sup>10</sup> (µg/g tobacco)	Present Method LOD (µg/g tobacco)	Present Method LRL (µg/g tobacco)
As	0.082	0.024	0.050 <sup>*</sup>
Be	0.0028	0.0030	0.0050 <sup>*</sup>
Cd	0.23	0.016	0.50 <sup>*</sup>
Co	0.05	0.018	0.100 <sup>*</sup>
Cr	0.16	0.088	0.20 <sup>*</sup>
Mn	13	2.85	20.0 <sup>*</sup>
Ni	0.14	0.217	0.50 <sup>*</sup>
Pb	0.16	0.012	0.20 <sup>*</sup>
Se	0.69	0.019	0.050 <sup>*</sup>
U	ND	0.0022	0.010 <sup>*</sup>

<sup>\*</sup> The LRLs for these analytes are significantly higher than the LODs, because the lowest standard concentrations were higher and were used as the LRLs.

ND = Not Determined

**Table 3**

Accuracy determined by analysis of standard and certified reference materials (n=20). Values are in µg/g dry tobacco or leaves.

	NIST 1570a	Target	NIST 1573a	Target	CTA-OTL1	Target	INCT-OBTL5	Target	INCT-PVTL6	Target	1S3	Previous <sup>(16)</sup>
Be	0.0071 ± 0.0008	NC	0.031 ± 0.003	NC	0.111 ± 0.005	NC	0.109 ± 0.007	0.0812 (info)	0.025 ± 0.002	NC	0.042 ± 0.003	0.044 ± 0.006
Cr	1.74 ± 0.07	NC	2.06 ± 0.12	1.99 ± 0.06	2.63 ± 0.16	2.59 ± 0.32	6.62 ± 0.50	6.30 (info)	0.861 ± 0.126	0.911 (info)	4.00 ± 0.27	3.63 ± 0.39
Mn	65.2 ± 1.5	75.9 ± 1.9	243 ± 7	246 ± 8	421 ± 9	412 ± 14	186 ± 4	180 ± 6	128 ± 8	136 ± 5	389 ± 9	391 ± 21
Co	0.36 ± 0.02	0.39 ± 0.05	0.55 ± 0.03	0.57 ± 0.02	0.949 ± 0.062	0.879 ± 0.039	0.962 ± 0.060	0.981 ± 0.067	0.154 ± 0.010	0.154 ± 0.007	1.46 ± 0.06	1.39 ± 0.10
Ni	2.16 ± 0.34	2.14 ± 0.10	1.67 ± 0.09	1.59 ± 0.07	5.97 ± 0.18	6.32 ± 0.65	8.44 ± 0.44	8.50 ± 0.49	1.35 ± 0.05	1.49 ± 0.14	2.78 ± 0.11	2.48 ± 0.16
Zn	0.066 ± 0.008	0.068 ± 0.012	0.113 ± 0.012	0.112 ± 0.004	0.546 ± 0.021	0.539 ± 0.060	0.767 ± 0.046	0.668 ± 0.086	0.128 ± 0.010	0.138 ± 0.010	0.309 ± 0.023	0.314 ± 0.028
As	0.115 ± 0.009	0.117 ± 0.009	0.062 ± 0.008	0.054 ± 0.003	0.038 ± 0.007	0.153 ± 0.018	0.044 ± 0.010	NC	0.020 ± 0.004	NC	0.061 ± 0.008	<0.69
Cd	2.70 ± 0.08	2.89 ± 0.07	1.42 ± 0.07	1.52 ± 0.04	1.18 ± 0.05	1.12 ± 0.12	2.85 ± 0.12	2.64 ± 0.14	2.40 ± 0.08	2.23 ± 0.12	1.44 ± 0.05	1.51 ± 0.11
Pb	0.18 ± 0.01	0.20 (info)	0.58 ± 0.03	NC	4.49 ± 0.27	4.91 ± 0.80	2.03 ± 0.07	2.01 ± 0.31	0.722 ± 0.040	0.972 ± 0.147	2.13 ± 0.09	1.98 ± 0.25
mean	0.146 ± 0.004	0.155 ± 0.023	0.038 ± 0.007	0.035 (info)	0.095 ± 0.005	0.10 (info)	0.101 ± 0.005	0.113 (info)	0.020 ± 0.001	0.022 (info)	0.074 ± 0.004	NC

NC = Not Characterized; Info = Information Value

Author manuscript; not certified by peer review; available in PMC 2016 June 01.

**Table 4**

Toxic metal concentrations in little cigar tobacco (n=5). Values are in µg/g dry tobacco.

	Be	Cr	Mn	Co	Ni	As	Se	Cd	Pb	U
Hav-A-Tampa	0.029 ± 0.003	6.46 ± 0.90	202 ± 15	0.80 ± 0.07	4.36 ± 0.45	0.17 ± 0.02	0.14 ± 0.02	1.24 ± 0.05	0.69 ± 0.05	0.040 ± 0.005
Muriel Sweets	0.024 ± 0.002	1.24 ± 0.30	138 ± 12	0.71 ± 0.05	1.58 ± 0.08	0.13 ± 0.01	0.31 ± 0.04	1.31 ± 0.05	0.70 ± 0.12	0.029 ± 0.004
Winchester	0.027 ± 0.006	0.88 ± 0.19	202 ± 6	0.68 ± 0.05	1.67 ± 0.15	0.14 ± 0.01	0.369 ± 0.031	1.69 ± 0.08	0.54 ± 0.06	0.029 ± 0.002
Vaquero	0.036 ± 0.001	1.58 ± 0.23	160 ± 13	0.81 ± 0.03	1.97 ± 0.26	0.55 ± 0.03	0.30 ± 0.01	1.06 ± 0.04	0.78 ± 0.05	0.056 ± 0.004
Santa Fe	0.033 ± 0.002	2.29 ± 0.16	139 ± 11	0.65 ± 0.03	2.80 ± 0.28	0.17 ± 0.01	0.10 ± 0.01	0.808 ± 0.027	1.00 ± 0.08	0.048 ± 0.006
Phillies (Regular)	0.020 ± 0.002	1.84 ± 0.53	146 ± 11	0.71 ± 0.07	2.29 ± 0.49	0.12 ± 0.01	0.33 ± 0.03	1.36 ± 0.02	0.78 ± 0.03	0.025 ± 0.001
Captain Black	0.029 ± 0.004	0.88 ± 0.19	210 ± 8	0.82 ± 0.04	1.50 ± 0.06	0.14 ± 0.01	0.30 ± 0.03	1.74 ± 0.13	0.46 ± 0.05	0.034 ± 0.002
Swisher Sweets	0.075 ± 0.010	3.34 ± 0.65	166 ± 20	0.84 ± 0.07	3.14 ± 0.29	0.26 ± 0.02	0.11 ± 0.02	0.910 ± 0.042	1.23 ± 0.16	0.113 ± 0.017
Cheyenne	0.037 ± 0.002	1.88 ± 0.14	156 ± 9	0.82 ± 0.03	4.37 ± 0.19	0.66 ± 0.03	0.24 ± 0.01	1.06 ± 0.03	0.81 ± 0.05	0.055 ± 0.005
Murano (Regular)	0.038 ± 0.003	1.26 ± 0.04	202 ± 8	0.98 ± 0.06	1.93 ± 0.10	0.16 ± 0.01	0.14 ± 0.01	1.21 ± 0.04	0.59 ± 0.02	0.055 ± 0.002
Clipper Black (Red)	0.025 ± 0.002	1.83 ± 0.05	374 ± 27	0.74 ± 0.07	1.69 ± 0.13	0.19 ± 0.01	0.18 ± 0.01	1.12 ± 0.04	0.57 ± 0.10	0.038 ± 0.002
Vendetta (9mm)	0.036 ± 0.003	1.33 ± 0.05	169 ± 14	0.86 ± 0.05	1.97 ± 0.07	0.28 ± 0.01	0.21 ± 0.02	1.17 ± 0.09	0.62 ± 0.03	0.051 ± 0.002
Al Capone (Sweets Filter)	0.038 ± 0.005	1.92 ± 0.19	225 ± 12	1.00 ± 0.08	2.97 ± 0.17	0.21 ± 0.03	0.090 ± 0.005	1.68 ± 0.09	0.74 ± 0.05	0.037 ± 0.003
Smoker's Best (Light)	0.047 ± 0.005	2.74 ± 0.17	177 ± 10	0.83 ± 0.02	3.13 ± 0.15	0.20 ± 0.01	0.11 ± 0.01	0.788 ± 0.040	0.910 ± 0.057	0.074 ± 0.004
Smoker's Best (Menthol)	0.045 ± 0.003	2.42 ± 0.14	176 ± 10	0.84 ± 0.05	2.96 ± 0.20	0.21 ± 0.01	0.13 ± 0.01	0.752 ± 0.035	0.88 ± 0.04	0.074 ± 0.006
Remington (Full Flavor)	0.032 ± 0.002	1.54 ± 0.17	166 ± 10	0.86 ± 0.04	2.08 ± 0.08	0.16 ± 0.01	0.21 ± 0.01	1.16 ± 0.07	0.59 ± 0.02	0.046 ± 0.002
Prime Time (Blueberry)	0.051 ± 0.004	2.75 ± 0.13	178 ± 10	0.90 ± 0.05	3.15 ± 0.25	0.22 ± 0.01	0.22 ± 0.01	1.20 ± 0.05	0.96 ± 0.04	0.077 ± 0.006